Targeted reactivation during sleep differentially affects negative memories in socially anxious and healthy children and adolescents

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Abstract: Cognitive models propose a negative memory bias as one key factor contributing to the emergence and maintenance of social anxiety disorder (SAD). The long-term consolidation of memories relies on memory reactivations during sleep. We investigated in SAD patients and healthy controls the role of memory reactivations during sleep in the long-term consolidation of positive and negative information. Socially anxious and healthy children and adolescents learnt associations between pictures showing ambiguous situations and positive or negative words defining the situations' outcome. Half of the words were re-presented during post-learning sleep (i.e. they were cued). Recall of picture-word associations and subjective ratings of pleasantness and arousal in response to the pictures was tested for cued and uncued stimuli. In the morning after cueing, cueing facilitated retention of positive and negative memories equally well in SAD patients and healthy controls. One week later, cueing led to reduced ratings of pleasantness of negative information in SAD but not in healthy controls. These findings were coincided by more pronounced EEG theta activity over frontal, temporal and parietal regions in response to negative stimuli in SAD patients. Our findings suggest that the preferential abstraction of negative emotional information during sleep might represent one factor underlying the negative memory bias in SAD. SIGNIFICANCE STATEMENT We aim to uncover mechanisms underlying the characteristic negative memory bias in social anxiety disorder (SAD). The formation of long-lasting memories - a process referred to as memory consolidation - depend on the reactivation of newly acquired memories during sleep. We could demonstrate that experimentally induced memory reactivation during sleep renders long-term memories of negative experiences more negative in SAD patients but not in healthy controls. In parallel, reactivating negative experiences was coincided by more pronounced oscillatory theta activity in these patients. These results provide first evidence that memory reactivation during sleep might contribute to the negative memory bias in SAD.

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Targeted reactivation during sleep differentially affects negative memories in socially anxious and healthy children and adolescents

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Targeted reactivation during sleep differentially affects negative memories in socially anxious and healthy children and adolescents

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Abstract

Cognitive models propose a negative memory bias as one key factor contributing to the emergence and maintenance of social anxiety disorder (SAD). The long-term consolidation of memories relies on memory reactivations during sleep. We investigated in SAD patients and healthy controls the role of memory reactivations during sleep in the long-term consolidation of positive and negative information. Socially anxious and healthy children and adolescents learnt associations between pictures showing ambiguous situations and positive or negative words defining the situations' outcome. Half of the words were re-presented during post-learning sleep (i.e. they were cued). Recall of picture-word associations and subjective ratings of pleasantness and arousal in response to the pictures was tested for cued and uncued stimuli. In the morning after cueing, cueing facilitated retention of positive and negative memories equally well in SAD patients and healthy controls. One week later, cueing led to reduced ratings of pleasantness of negative information in SAD but not in healthy controls. These findings were coincided by more pronounced EEG theta activity over frontal, temporal and parietal regions in response to negative stimuli in SAD patients. Our findings suggest that the preferential abstraction of negative emotional information during sleep might represent one factor underlying the negative memory bias in SAD.

Significance Statement

We aim to uncover mechanisms underlying the characteristic negative memory bias in social anxiety disorder (SAD). The formation of long-lasting memories – a process referred to as memory consolidation - depend on the reactivation of newly acquired memories during sleep. We could demonstrate that experimentally induced memory reactivation during sleep renders long-term memories of negative experiences more negative in SAD patients but not in healthy controls. In parallel, reactivating negative experiences was coincided by more pronounced oscillatory theta activity in these patients. These results provide first evidence that memory reactivation during sleep might contribute to the negative memory bias in SAD.

Introduction

Social anxiety disorder (SAD) is one of the most common mental disorders with a lifetime prevalence of up to 5-13% of the general population (Essau et al., 1999; Furmark, 2002). SAD typically emerges during early to late adolescence and 75% of anxious adults already met the criteria of an anxiety disorder during childhood and adolescence (Pollack et al., 1996; Beesdo et al., 2009). These findings clearly indicate that there is a great need to improve our understanding of the aetiology and course of social anxiety in youth.

Cognitive models propose that superior memory for negative experiences is one major factor contributing to the emergence and maintenance of anxiety disorders (Clark and Wells, 1995; Rapee and Heimberg, 1997). However, empirical research on memory biases in SAD reveals inconsistent results. That is, a few studies report a bias towards negative threat-related memories (Coles and Heimberg, 2005; Foa et al., 2000; Lundh and Öst, 1996) while others report either no bias (Cloitre et al., 1995) or a memory bias only for implicit but not explicit memories (MacLeod and McLaughlin, 1995; Becker et al., 1999). A limiting factor in these experiments might be the length of the retention interval since the majority of these studies tested retention performance only a few minutes after encoding. In fact, memory biases might develop over longer time periods, as all newly acquired memory traces are subject to consolidation processes that start after encoding and take at least hours to days (Dudai et al., 2015). In the process of long-term consolidation, newly acquired memories are assumed to be gradually integrated into neocortical networks of existing memories (McGaugh, 2000; Dudai, 2004). Importantly, the long-term consolidation of memories does not only result in a stabilization of a memory trace but it undergoes transformation processes and can also result in the extraction of gist information (i.e., the extraction of core information from experiences; Nadel et al., 2012; Winocur et al., 2010; Inostroza and Born, 2013; Brainerd and Reyna, 2001).

Sleep after learning critically benefits the consolidation of newly acquired neutral and emotional information during childhood, adolescence and adulthood (for review see Rasch and Born, 2013; Wilhelm et al., 2012). Besides the mere stabilization of the newly acquired information, sleep has been shown to benefit the extraction of gist information (Gomez et al., 2006; Fischer et al., 2006; Wilhelm et al., 2013; Durrant et al., 2011; Stickgold and Walker, 2013; Payne et al., 2008). Sleep-dependent consolidation of memories critically relies on the repeated reactivation of these memories (Rasch and Born, 2013). In humans, this was demonstrated in a number of experiments using the method of context cueing. Re-exposing odours, words or tones (i.e. cues) during sleep that are associated with newly learnt neutral or emotional memories induced the reactivation of these memories and benefited their later recall (Rasch et al., 2007; Rudoy et al., 2009; Cairney et al., 2014; Hauner et al., 2013; Groch et al., 2016). The beneficial effect of memory cueing during sleep is represented on the electrophysiological level by an increase in EEG oscillatory theta (5-8 Hz) and spindle activity (10-15 Hz) (Schreiner and Rasch, 2015; Schreiner et al., 2015; Lehmann et al., 2016). In the present study, we investigated in SAD patients and healthy controls the impact of memory cueing during post-learning sleep on the consolidation of newly acquired positive and negative memories. We hypothesized that in SAD patients, cueing preferentially affects newly encoded negative experiences, especially when tested after long retention intervals.

Materials and Methods

Participants. Thirteen children and adolescents suffering from social anxiety disorder (SAD; mean ± SEM: 13.36 ± 0.57 years; 8 females, 5 males) and thirteen healthy age-matched children and adolescents (mean ± SEM: 13.15 ± 0.57 years; 4 females, 9 males) participated in the study. Healthy participants were recruited via advertisements in schools and local newspapers and socially anxious participants were recruited at the Child and Adolescent Psychiatry and Psychotherapy, University of Zurich, Switzerland. SAD patients met the

criteria for a social anxiety disorder according to the criteria of the DSM-V (American Psychiatric Association, 2013) evaluated by an experienced Child and Adolescent Psychiatrist. To confirm initial clinical diagnosis, a clinical interview was performed by a second experienced Child and Adolescent Psychiatrist using the Mini-International Neuropsychiatric Interview (M.I.N.I.-KID, Sheehan et al., 1998). To exclude prevalent comorbidity of depression in these patients the „Children's Depression Rating Scale Revised“ (CDRS-R; Keller et al., 2011; see Table 1 for results on this interview) was performed. Symptoms of social anxiety were assessed in all participants using two questionnaires, i.e. the „Sozialphobie und Angstinventar für Kinder“ (SPAIK; Melfsen et al., 2001) as well as the „Social Anxiety Scale for Children Revised“–Child and Parents version (SASC-R-D, Melfsen and Florin, 1997, see Table 1 for results on these questionnaires). One participant who was originally planned to be included in the group of healthy participants was assigned post-hoc into the group of socially anxious participants because of high scores of social anxiety as indicated by the SASC-R-D (more than two standard deviations above the group mean). Interviews with all participants and their parents, as well as questionnaires, ensured that the participants had no cognitive impairments, neurological or sleep disorders. Participants did not take any medication at the time of the experiment except for one patient who received a selective serotonin reuptake inhibitor. Note, that excluding this participant as well as the participant who was assigned post-hoc to the SAD group and their age-matched controls did not change the main findings (i.e. interaction ‘cueing’ x ‘group’ in a 2 x 2 ANOVA of pleasantness ratings for negative stimuli in Test Session 2 as well as the respective post-hoc t-test were both $p < 0.05$). The ingestion of caffeine or alcohol was not allowed on experiment days. The intelligence quotient (IQ) was assessed (WISC-IV; Petermann and Petermann, 2011) in each participant. Only participants with an IQ between 85 and 135 were included in this experiment. Socially anxious patients did not differ from healthy controls with regard to IQ ($p > 0.38$, see Table 1). All participants were asked for individual sleep habits, i.e., usual

time to go to bed, time getting up, etc. in order to schedule both nights in the sleep laboratory in accordance with their usual sleep habits. One participant from the group of healthy participants did not enter the sleep EEG analyses because of technical problems.

The study was approved by the local ethics committee. The parents of the participants gave written informed consent before their childrens' participation. Additionally, all children and adolescents provided verbal assent. All participants received a voucher for reimbursement of expenses.

Design and Procedure. Please see Figure 1A for an illustration of the design and procedure. Participants were adapted to polysomnographic recordings on a night preceding the experiment proper. Experimental and adaptation night in the sleep lab were separated by at least one night at home to exclude possible effects of bad sleep quality in the adaptation night on sleep in the experimental night. In the experimental night, participants came to the sleep lab around 2.5 hrs before participants' habitual bedtime. First, the electrode net was placed in order to record nocturnal sleep during the subsequent night. Thereafter, participants performed the picture-word association task (Learning Session) and went to bed afterwards. During NREM sleep, half of the learnt words was presented via loudspeaker placed behind the participants' head to stimulate the reactivation of respective picture-word associations. Testing started ~45 minutes after waking up in the morning to avoid any modulating effect of sleep inertia on recall performance. This Test Session 1 as well as the Test Session 2 one week later included the memory recall of picture-word associations and, in a second run, subjective ratings on the level of arousal and pleasantness of pictures on a 9-point self-assessment-manikin (SAM, Bradley and Lang, 1994, see also later paragraph for a detailed description of the rating procedure).

Sleep EEG. Sleep in the experimental and the adaptation night was recorded using high-density sleep EEG (Electrical Geodesics Sensor Net for long-term monitoring, 128 channels, referenced to a vertex electrode) including electromyographic and electrooculographic recordings. Data were sampled at 500 Hz (0.01–200 Hz). Offline, the EEG was bandpass filtered (0.5–50 Hz) and downsampled to 128 Hz. The EEG was visually scored for sleep stages Wake, N1, N2, N3 and REM sleep at frontal, central and occipital electrodes (20 s epochs) based on American Academy of Sleep Medicine standard criteria (Iber et al., 2007). Socially anxious and healthy adolescents did not differ in any of the relevant sleep parameters (see Table 2 for descriptives).

Memory cueing during sleep. During post-learning periods of NREM sleep, emotional words that had been paired with a picture during the learning phase were presented again auditorily via loudspeaker (with a 55 dB sound pressure level). Half of the learnt words were cued, whereas the other half the learnt words were not cued. Half of the cued words were randomly and individually chosen from the group of words that had been correctly remembered by an individual during the learning phase (i.e. hits), whereas the other half was taken from unknown words (i.e. misses). Half of the cued words were positive and half of them were negative. For example, given that a participant correctly remembered half of the 120 picture-word associations- with half of them being negative and half of them being positive—this would result in cueing of 15 positive and 15 negative hits and misses. Words were presented with an inter-stimulus-interval of 6 seconds with a random jitter of 0 to 0.4 seconds. Each of the chosen words was cued at a maximum of 12 times (mean number of positive cues: SAD – 268.92 ± 21.75, healthy controls – 223.92 ± 15.92; negative cues: SAD – 267.33 ± 22.01, healthy controls – 223.69 ± 16.05; all $p > 0.10$). An experienced experimenter inspected the EEG in real-time to determine sleep stages and to detect any indicator of an arousal. Word cueing was started when a participant had spent more than 10 minutes in non-REM stage N3,

and it was immediately stopped whenever any sign of an arousal, waking up or any other change in sleep stages was observed by the experimenter. In line with previous studies showing beneficial effects of cueing on memory consolidation during NonRem (Schreiner et al., 2015; Schreiner and Rasch, 2015; Lehmann et al., 2016a) but not during REM (Lehmann et al., 2016a) memory cues were presented solely during NonRem sleep in this study.

Picture-word association task. The picture-word association task is based on previous studies that aimed to induce mental imagery using picture–word cues (Holmes et al., 2008; Pictet et al., 2011; see Figure 1B for an illustration of the task). This specific task was used because 1) mental imagery of picture word-associations has been shown to evoke strong emotional responses with this being attributed to the personal relevance of the memories (Holmes et al. 2008; Pictet et al. 2011) and 2) picture-word associations have been found to be sensitive for sleep-dependent processes of memory consolidation (Lehmann et al., 2016b) as well as for the beneficial effects of memory cueing during sleep (Lehmann et al., 2016a). The task includes a set of 120 (for participants older than 12.4 yrs) or 102 (for participants younger than 12.4 yrs) ambiguous photographs of common everyday life objects and scenes. Pictures were paired with words that had been chosen so that their combination with the picture (e.g. a picture showing a person acting in a play in front of an audience) either suggests a positive (half of the pictures; e.g. "applause") or a negative outcome of the scene shown on the picture (e.g. “jeer”). Before starting the learning procedure, all participants were instructed in an imagery generation task similar to those used in previous studies (e.g. Holmes et al., 2008) and underwent two practice trials. Participants were also instructed to memorize the picture-word associations because memory performance would be tested after learning and again after the retention interval. During learning, each of the picture-word associations was presented for 1500ms. A black screen then appeared for 3000ms during which the participants were asked to shut their eyes and to generate mental imageries about the event. Thereafter, a

1000ms beep informed participants to open their eyes and the next picture-word association was presented. Memory performance was tested for all picture-word associations in a recall procedure immediately after learning to assess baseline performance, and with the same procedure again the next morning and one week later. In these recall tests, all pictures were presented one after another in a random order for a duration of 1500ms and the participants were required to recall the associated word (by speaking out loud the remembered word to the experimenter who stood next to them and recorded memory performance). The time to generate a response was not limited. In Test Session 2, one week later, all participants were called by telephone by the experimenter and received via mail a slide show containing all pictures they had seen during the experiment. All words that were identical with the learnt word or included the word-stem of the learnt word were counted as correct (i.e. success and succeed). In case the subject came up with a number of words, they were prompted to choose one. We calculated the relative difference between correctly recalled words before sleep and i) in the morning after sleep (Test Session 1) and ii) one week later (Test Session 2) for cued and un-cued picture-word associations as a measure of retention performance (i.e., 100% means the same amount of words being correctly recalled in both Test Sessions).

Ratings of pleasantness and arousal. To assess the two emotional dimensions, i.e. pleasantness and arousal (i.e. excitement), we used the SAM rating system (Bradley and Lang, 1994). SAM is a nonverbal instrument for the assessment of emotional responses to a stimulus which has been found to be suitable for this purpose in previous studies using IAPS pictures (e.g., Hamm et al., 1993; Groch et al., 2013; Wagner et al., 2002). After being tested on their memory performance for picture-word associations, participants were instructed that they will see again each of the pictures and that they had to rate spontaneously and quickly how emotional they perceive the picture on the dimensions of pleasantness and arousal using a 9-point scale (1 = very unpleasant, 5 = neutral, 9 = very pleasant; 1 = not arousing at all, and

9 = very arousing, respectively). In order to do so, they had to use the buttons 1 to 9 of the computer keyboard in Test Session 1. In Test Session 2, they had to give their ratings verbally to the experimenter via telephone while viewing the pictures.

Analyses of power changes in response to cues.

EEG signals were preprocessed using Brain Vision Analyzer 2.0 (Brain Products, Gilching, Germany). Preprocessing included re-referencing of the raw EEG data to the average of the two mastoids and low-pass (30 Hz, roll-off 24 dB per octave) and high-pass filtering (0.1 Hz, roll-off 12 dB per octave). EEG data were epoched into segments of 2 s before to 3 s after word onset. For each individual, segments were categorized in stimuli that were and stimuli that were not remembered in the Test Session in the morning (i.e. referred to as "later remembered" and "later forgotten" stimuli). Succeeding EEG oscillatory analyses were performed using the open source Fieldtrip toolbox (Oostenveld et al., 2011) running on Matlab R2014a (MathWorks, Natick, MA). Time-frequency analysis was computed for each trial by using a 7-cycle Morlet wavelet decomposition, ranging from 2 to 25 Hz in 0.5 Hz steps. A sliding window with a step size of 10 ms was applied across the entire length of the epochs. Single trials were normalized with respect to a pre-stimulus time window ranging from -1000 ms to -100 ms.

Statistical analyses. Statistical analysis of memory retention and subjective ratings was based on a 2 x 2 x 2 x 2 analyses of variance (ANOVA) including the repeated measures factors 'valence' (positive, negative), 'cueing' (cued, un-cued) and 'time' (Test Session 1, Test Session 2) and the between-subjects factor 'group' (socially anxious, healthy controls). In case of significant interactions in this initial analysis, 2 x 2 ANOVAs either including the factors 'valence' and 'cueing' or 'valence' and 'group' were calculated. Post-hoc comparisons were performed using students t-tests. The level of significance was set to $p \leq 0.05$.

Statistical analyses of the EEG data were performed with a nonparametric randomization test using cluster correction as implemented in FieldTrip, (Maris and Oostenveld, 2007). The cluster alpha was set to 0.025 and 500 randomizations were conducted for all tests. Clusters were considered significant at $p < 0.05$ (two-sided).

Results

Retention of picture-word associations and ratings of pleasantness

In the evening before sleep, socially anxious patients (SAD) and healthy controls learnt associations between pictures and positive and negative words equally well (main effect of 'valence': $F(1,24) = 1.66$, $p = 0.20$ in a 2 (valence) x 2 (group) x 2 (cueing) ANOVA). Retention rates in this Learning Session did not differ between socially anxious patients and healthy controls (main effect of 'group' and interaction 'valence' x 'group', both $p = 0.42$).

During post-learning sleep, half of the positive and half of the negative words that had been associated with ambiguous pictures in the Learning Session were acoustically presented again (being termed as "cued" associations), whereas the other half was not presented ("un-cued" associations). We analyzed whether cueing did substantially strengthen memory for picture-word associations in the morning after sleep (Test Session 1) and one week later (Test Session 2) in a 2 (time) x 2 (cueing) x 2 (valence) x 2 (group) ANOVA. Importantly, the effect of cueing on memory recall was significantly modulated by the time of testing and the group of participants (interaction 'time' x 'group' x 'cueing': $F(1,24) = 4.26$; $p = 0.05$). To disentangle the effect of cueing on memory recall at the different time points, we analysed both test sessions separately in two 2 (cueing) x 2 (group) x 2 (valence) ANOVAs. In Test Session 1, both groups correctly remembered more picture-word associations that had been cued during post-learning sleep than those that had not been cued (main effect of 'cueing': $F(1,24) = 9.08$, $p = 0.006$; see Table 3 for data on memory recall). Importantly, the beneficial

effect of memory cueing during sleep on retention performance did not differ between healthy and socially anxious children and adolescents (interaction 'group' x 'cueing': $F(1,24) = 0.001$, $p = 0.98$) and it was also not modulated by the valence of picture-word pairs (interaction 'cueing' x 'valence': $F(1,24) = 1.79$, $p = 0.19$). When tested one week later (Test Session 2), cued stimuli were no longer better remembered than un-cued stimuli (main effect of 'cueing': $F(1,24) = 0.1$, $p = 0.75$; interaction 'cueing' x 'valence': $F(1,24) = 0.34$, $p = 0.57$). At this time, there was a marginal significant interaction between the factors 'group' and 'cueing' ($F(1,24) = 4.09$, $p = 0.055$) suggesting that cueing differentially affects memory performance in socially anxious and healthy children and adolescents. However, further 2 (cueing) x 2 (valence) ANOVAs separately for both groups did not reveal any impact of cueing on memory recall (SAD: main effect of 'cueing': $F(1,12) = 2.20$, $p = 0.16$; interaction 'cueing' x 'valence': $F(1,12) = 0.60$, $p = 0.46$; HC: main effect of 'cueing': $F(1,12) = 1.92$, $p = 0.19$; interaction 'cueing' x 'valence': $F(1,12) = 0.003$, $p = 0.96$).

Subjective ratings of pleasantness of pictures

In a next step, we analyzed whether cueing modulates the subjectively rated pleasantness of pictures. A 2 (time) x 2 (cueing) x 2 (valence) x 2 (group) ANOVA revealed that cueing substantially affects pleasantness with this being modulated by the group of participants (interaction 'group' x 'cueing': $F(1,24) = 5.66$, $p = 0.026$) as well as by the time of testing and the valence of stimuli (interaction 'cueing' x 'valence' x 'time': $F(1,24) = 5.15$; $p = 0.033$, see Table 3 for descriptive data on emotional ratings). In order to further elucidate this complex interaction between the different factors, we again analysed the two test sessions separately in two 2 (cueing) x 2 (group) x 2 (valence) ANOVAs. The valence of words modulated later ratings of pleasantness of the associated pictures in both test sessions (main effect of 'valence', both $p = 0.001$). These results indicate that our experimental manipulation (i.e. to

disambiguate pictures by associating a positive or a negative word) was successful insofar as those pictures that had been associated with a positive word were rated as being more pleasant than those pictures that had been associated with a negative word. Subjectively rated pleasantness was neither affected by cueing nor by the mental health of participants in the morning after cueing (all main effects and interactions, $p > 0.11$; Figure 2A; note that Figure 2 depicts the difference of pleasantness and arousal ratings between cued and un-cued items and results from subtracting the mean values for cued and un-cued items that can be seen in Table 3). Importantly, one week later (Test Session 2), cueing substantially modulated ratings of pleasantness in accordance with the valence of the associated word (interaction 'cueing' x 'valence', $F(1,24) = 8.00$, $p = 0.009$; Figure 2A). Separate 2 (cueing) x 2 (group) ANOVAs for positive and negative stimuli revealed a significant effect of cueing on rated pleasantness of positive stimuli (main effect of 'cueing': $F(1,24) = 5.83$, $p = 0.024$). The effect of cueing did not significantly differ between socially anxious and healthy participants (interaction 'cueing' x 'group:' $F(1,24) = 2.53$, $p = 0.12$). Although this interaction, did not reach significance, we calculated further post-hoc analysis for exploratory reasons. These t-tests indicated that in healthy controls cueing significantly increased ratings of pleasantness for pictures previously being associated with a positive word ($t(12) = 2.88$, $p = 0.014$; Figure 2A) while this was not the case in socially anxious participants ($t(12) = 0.58$, $p < 0.57$). For negative stimuli, the effect of cueing on the rated pleasantness significantly differed between both participants' groups (interaction 'cueing' x 'group': $F(1,24) = 4.63$, $p = 0.042$). Post-hoc t-tests revealed a significant effect of cueing in socially anxious ($t(12) = 2.3$, $p = 0.04$; Figure 2A) but not in healthy participants ($t(12) = 0.53$, $p = 0.60$).

Subjective ratings of arousal of pictures

Memory cueing during post-learning periods of sleep significantly affected the arousal ratings of pictures and this was modulated by the time of testing and the group of participants (interaction 'time' x 'group' x 'cueing': $F(1,24) = 4.72$; $p = 0.04$ in a 2 (time) x 2 (cueing) x 2 (valence) x 2 (group) ANOVA). Further 2 (cueing) x 2 (valence) x 2 (group) ANOVAs were calculated for the two test sessions separately. In the morning after cueing (Test Session 1), the subjectively rated arousal of pictures was higher for those pictures that had been associated with a negative word as compared to the pictures associated with a positive word (main effect of 'valence': $F(1,24) = 8.50$, $p = 0.008$; see Table 3). However, at this time, this effect of valence was different between SAD and healthy controls as indicated by a significant interaction between the factor 'group' and 'valence' ($F(1,24) = 7.40$, $p = 0.012$). 2 (cueing) x 2 (valence) ANOVAs separately for both groups revealed that socially anxious but not healthy controls rated pictures previously associated with a negative word to be more arousing than those pictures associated with a positive word (main effect of 'valence' in SAD: $F(1,12) = 10.18$, $p = 0.008$; in healthy controls: $F(1,12) = 0.04$, $p = 0.83$). One week later (Test Session 2), socially anxious participants rated positive and negative stimuli to be more arousing than healthy participants (main effect of 'group': $F(1,24) = 4.53$, $p = 0.044$). In contrast to Test Session 1, in Test Session 2, arousal ratings no longer differed between positive and negative stimuli (main effect of 'valence': $F(1,24) = 1.24$, $p = 0.27$). However, one week after cueing (Test Session 2), arousal ratings were affected by cueing the respective information during post-learning sleep as indicated by the three-way interaction between the factors 'valence' x 'cueing' x 'group' ($F(1,24) = 4.85$, $p = 0.038$). More specifically, cueing reduced the subjectively rated arousal of pictures that previously had been associated with a negative word in socially anxious but not in healthy participants (cued vs. un-cued – SAD $t(12) = 2.3$, $p = 0.04$; healthy controls $t(12) = 0.07$, $p = 0.94$; Figure 2B). For pictures associated with a positive word, cueing neither modulated subjective arousal ratings in SAD patients nor in healthy controls (both $p > 0.12$; Figure 2B).

Because previous studies indicate a beneficial effect of slow wave sleep on processes of memory consolidation (Marshall et al., 2006; Ngo et al., 2013), we correlated this sleep parameter with memory retention and ratings of pleasantness and arousal. Neither the absolute nor relative amount of slow wave sleep correlated with the effect of cueing on any of these measures (all $p > 0.21$).

Neuronal correlates of memory cueing during sleep

In order to uncover possible electrophysiological correlates of the observed behavioural differences between socially anxious and healthy participants, the induced oscillatory responses to cues during post-learning periods of sleep were analyzed. We calculated the subsequent memory effect (SME, Paller and Wagner, 2002), i.e. the difference between cues associated with later remembered and cues associated with later forgotten stimuli for positive and negative stimuli. Based on previous findings demonstrating a causal role of theta in the successful reactivation of memories (Schreiner and Rasch, 2015; Schreiner et al., 2015; Lehmann et al., 2016) these analyses were restricted to the theta frequency band (i.e. 5 - 8 Hz) in a time window of 600-800 ms after cue onset. The SME for negative cues showed pronounced differences between socially anxious and healthy participants in the theta frequency range (Fig. 3B) while this was not the case for positive cues (Fig. 3A). More specifically, the SME differed significantly between both groups of participants in an electrode cluster mainly spanning the left frontal, temporal and parietal cortex ($P = 0.002$, corrected for multiple comparisons; Fig. 3C). In order to uncover the origin of this significant interaction effect for negative stimuli, we contrasted theta activity in response to later remembered and later forgotten negative cues in both groups of participants separately. In socially anxious participants, later remembered as compared to later forgotten negative cues induced higher theta activity in an electrode cluster over the left temporal and parietal cortex

(P = 0.009, corrected for multiple comparisons; Fig. 3D). In healthy controls, comparing later remembered as compared to later forgotten negative cues revealed reduced theta activity in a cluster mainly located over the left frontal and temporal cortex (P = 0.012, corrected for multiple comparisons; Fig. 3E).

Discussion

Here, we found in children and adolescents with social anxiety disorder (SAD) and healthy controls that memory cueing during post-learning sleep benefits recall of positive and negative memories in the morning after cueing. One week later, cueing reduced subjectively rated pleasantness of pictures previously being associated with a negative word in SAD but not in healthy controls. At the same time, the subjectively rated arousal of these negatively disambiguated pictures was reduced for cued compared to un-cued negative stimuli in socially anxious but not healthy youth.

Our findings of increased retention performance for cued as compared to un-cued stimuli in the morning after cueing is in line with a number of previous studies reporting beneficial effects of cueing on the consolidation of neutral memories (Rasch et al., 2007; Rudoy et al., 2009; Groch et al., 2016; Schönauer et al., 2014). Moreover, our findings support first empirical evidences indicating that cueing can facilitate the consolidation of emotional material as well (Cairney et al., 2014; Lehmann et al., 2016). However, in our study this memory superiority of cued stimuli was no longer evident one week later. In contrast, ratings of pleasantness were modulated by memory cueing not immediately after sleep but one week later. These different time scales might be related to the unique nature of both measures of emotional memory processing. In our task, participants learnt associations between initially ambiguous pictures and words that had been chosen so that their combination either suggests a positive or a negative outcome of the picture. Importantly,

during encoding they had to form vivid mental images in response to the picture-word cues by seeing the scene through their own eyes as if they were actively involved thereby including own thoughts, emotions, and various kinds of sensations. The exact word associated with the picture is rather a detail of this rich episode while ratings of pleasantness of the picture can be interpreted as a measure of gist in this context. This is well in line with previous assumptions indicating that pleasantness is one of the most fundamental gist representations of a stimulus (Reyna and Brainerd, 1998; Chen and Bargh, 1999; Rivers, Reyna and Mills, 2008). Memory consolidation is a time-consuming process leading to fundamental changes in the neural representation that are accompanied by significant changes in the nature of the memory (Brainerd and Reyna, 1991; Frankland and Bontempi, 2005; Inostroza and Born, 2013; Nadel and Moscovitch, 2001;Winocur et al., 2010). It is assumed that repeatedly reactivating hippocampal memory traces together with associated memory contents in the neocortex results over time in the integration of new memories in neocortical networks while the gist of the memory is extracted (Nadel et al., 2012; Inostroza and Born, 2013). Memory cueing in the post-learning night induces the reactivation of memories in the hippocampus (Rasch et al., 2007; Diekelmann et al., 2011) and this led in the next morning to improved retention of picture-word associations. The immediate stabilization of hippocampal memory traces might have facilitated the long-lasting process of extracting gist information from these memory traces. Support for the notion that gist abstraction is relatively slow comes from studies investigating the effect of sleep on unbinding of an item from its associated context which is another phenomena resulting from the redistribution of memories from hippocampal to neocortical regions. A “decontextualisation” of memories was found after three but not after one night of sleep (Deliens et al., 2013; Deliens et al., 2014; Jurewicz et al., 2016). Moreover, Gais et al. (2007) found that sleep after learning novel words led to an increase in hippocampal activation during recall of the words two days later and in an increase in mPFC activity after six months. Together these findings indicate that the redistribution of memory

453 traces from the hippocampus to neocortical networks that underlies the extraction of gist
454 information might be initiated in the first night after learning but is observable at the
455 behavioural level not before additional nights with further endogenous reactivation having
456 taken place.

457 Our findings suggest that cueing benefited the extraction of emotional gist information
458 from negative stimuli in SAD patients but not in healthy control. We hypothesize that these
459 findings are related to different pre-existing associative knowledge networks in SAD patients
460 and healthy controls. Information that matches pre-existing memory networks is assumed to
461 be integrated into neocortical memory networks more rapidly than non-matching information
462 (Tse et al., 2007; Van Kesteren et al., 2012; Ghosh and Gilboa, 2014; Brod et al., 2013).
463 Socially anxious patients typically recall more negative autobiographical memories, negative
464 self-defining memories and early adverse social events (Krans et al., 2014; Morgan, 2010)
465 indicating an elaborated network of negative memories in these patients. Activating this rich
466 negative memory network by presenting associated memory cues might have facilitated the
467 extraction of the emotional gist from the cued experiences. Our finding of greater theta
468 activity in response to negative cues in SAD patients as compared to healthy controls supports
469 this idea since theta oscillatory activity was found to be increased in response to cues being
470 capable to successfully induce memory reactivations (Schreiner and Rasch, 2015; Schreiner et
471 al., 2015). In contrast to our findings in socially anxious patients, in healthy controls, we
472 found a reduction of theta activity in response to remembered as compared to forgotten
473 negative picture-word associations. The majority of these associations might not have
474 matched very well for these individuals (for example a picture showing a person acting in a
475 play in front of an audience together with the word “jeer” might not match very well for a
476 healthy participant especially when he/she had to mentally imagine this scene on a field
477 perspective). It can be speculated that more obvious associations between pictures and words
478 need to be suppressed in order to learn less obvious associations. This might be reflected by

479 decreased frontal theta for remembered as compared to forgotten picture-word associations.
480 This assumption is in line with a previous study showing reduced theta associated with
481 beneficial memory formation specifically in a condition where item and context does not
482 match (Staudigl and Hanslmayer, 2013). Importantly, future studies should include measures
483 of "congruency" to assess the extent of picture-word fit on an individual level and to relate
484 this measure to theta activity in respone to the cues.

485 Our findings of higher arousal ratings irrespective of valence and cueing in socially
486 anxious as compared to healthy participants one week after encoding are in line with previous
487 studies demonstrating that the subjectively rated arousal as well as the arousal-induced
488 amygdala activation in response to emotional stimuli is higher in SAD patients as compared
489 to healthy controls (Brühl et al., 2014; Wieser and Moscovitch, 2015). Interestingly, memory
490 cueing during sleep resulted in reduced arousal ratings of negative stimuli in SAD patients.
491 This finding is consistent with a recent study in healthy adults reporting that cueing led to a
492 reduction of arousal ratings (Rihm and Rasch, 2015). It also fits well to the notion that the
493 consolidation of emotional memories not only results in the strengthening of memories (as
494 reflected by a decrease in pleasantness ratings for negative stimuli in our study) but also in the
495 reduction of their affective tone (Walker and van der Helm, 2009; Van der Helm al., 2011;
496 Gujar et al., 2011).

497 We would like to list three limitations of our experiment. First, because we only
498 examined memory cueing during sleep, but not during wakefulness, we cannot conclude that
499 the observed effect of memory cueing is specific to sleep. However, on the background of a
500 number of studies showing no or even detrimental effects of cueing on memory consolidation
501 during wakefulness (Rasch et al., 2007; Schreiner and Rasch, 2015; Diekelmann et al., 2011)
502 comparable effects after cueing during wakefulness appear to be unlikely. Second,
503 participating in an experiment that includes cognitive testing as well as an overnight stay in a
504 sleep laboratory is a huge challenge for socially anxious children and adolescents. On the one

hand, this can explain the rather low number of participants who were in the end willing to participate while a much larger number of patients (N > 90) were informed about the experiment but did not agree to participate. On the other hand, this challenging situation might have resulted in a selection of socially anxious participants with rather low to moderate symptom severity. However, we do not believe that a possible selection of socially anxious patients with relatively low level of symptom severity does challenge the interpretation of our findings. To the contrary, in more severely affected SAD patients we would expect even more pronounced differences. Third, both Test Sessions took place in different contexts. Test Session 1 was conducted in the sleep laboratory and participants were alone in the room while they rated the emotionality of pictures on the computer. In Test Session 2, participants were called by the experimenter and received via mail a slide show containing all previously seen pictures. They gave their ratings verbally to the experimenter. This situation might have induced higher general levels of arousal than in the sleep laboratory. Importantly, these differences cannot explain the observed cueing-induced changes in affective ratings as a higher general arousal should have equally affected cued and un-cued stimuli. However, it could explain the higher arousal ratings of pictures in Test Session 2 as compared to Test Session 1. According to the two-factor theory (Schachter and Singer, 1962) high arousal ratings might be caused by participants' misleadingly attributing the high arousal induced by the specific test context to the content of pictures.

Our findings have important implications for future research on normal and pathological processes of memory consolidation. First, to understand normal and pathological processes of memory consolidation it is crucial to follow a memory trace for a longer time than a few minutes or hours as the memory trace is subject to long-lasting consolidation and transformation processes. Second, cueing during sleep is an elegant tool to further improve our understanding of the pathological processes of emotional memory processing by providing information about the sensitivity of a network to respond to memory cues. In this

context, the role of preexisting memories or schemas in the consolidation of new experiences in mental disorders is highly relevant and needs further investigation. Third, emotional memory biases are observable early in the development of SAD, i.e. during adolescence, underpinning the need to further investigate memory processes at this early developmental period.

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716 **Figure legends**

717

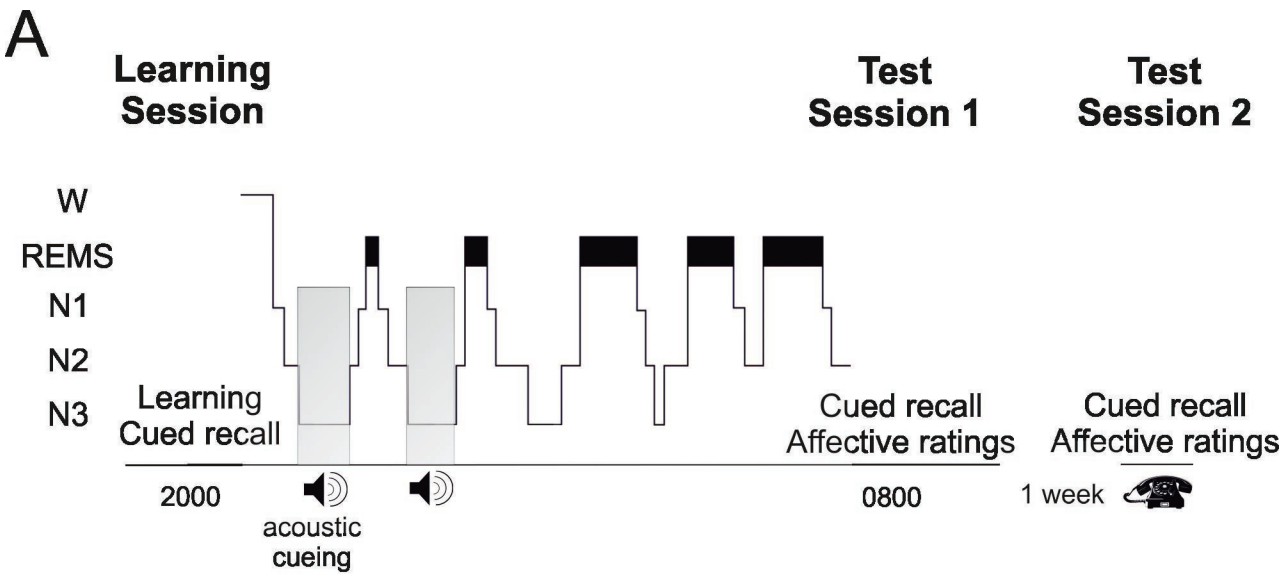
718 **Fig. 1.** Experimental procedure and picture-word association task. **(A)** After placement of the
719 electrode net, participants completed a learning phase followed by immediate recall testing
720 taking place between ~2000 - 2200 hrs. A typical polysomnogram visualizes the proportion of
721 sleep stages during the nocturnal retention interval (wake (W), non-rapid eye movement
722 (NonREM) sleep stages 1–3 (N1–N3), REMS) and speakers indicate that acoustic cueing was
723 performed during NonRem sleep in the post-learning night. The next morning (Test Session
724 1) and one week later (Test Session 2), participants performed a cued memory recall test and
725 rated subjectively perceived pleasantness and arousal of the pictures. **(B)** Learning the picture-
726 word association task required participants to associate ambiguous pictures with acoustically
727 presented positive or negative words that define the outcome of the pictures. Participants had
728 to vividly imagine themselves in the situation shown on the picture. In the cued memory
729 recall, pictures were shown again one after another and participants had to recall the
730 associated word. In a separate run, participants were required to indicate their emotional affect
731 elicited by each picture on a 9-point self-assessment manikin (SAM) rating scale on the
732 dimensions of pleasantness and arousal.

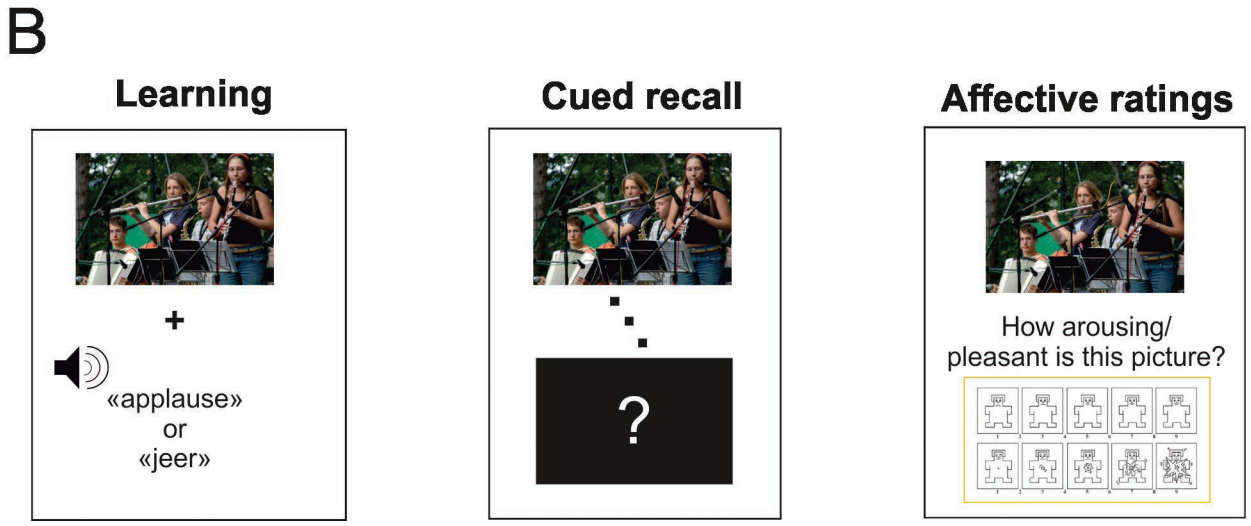
733

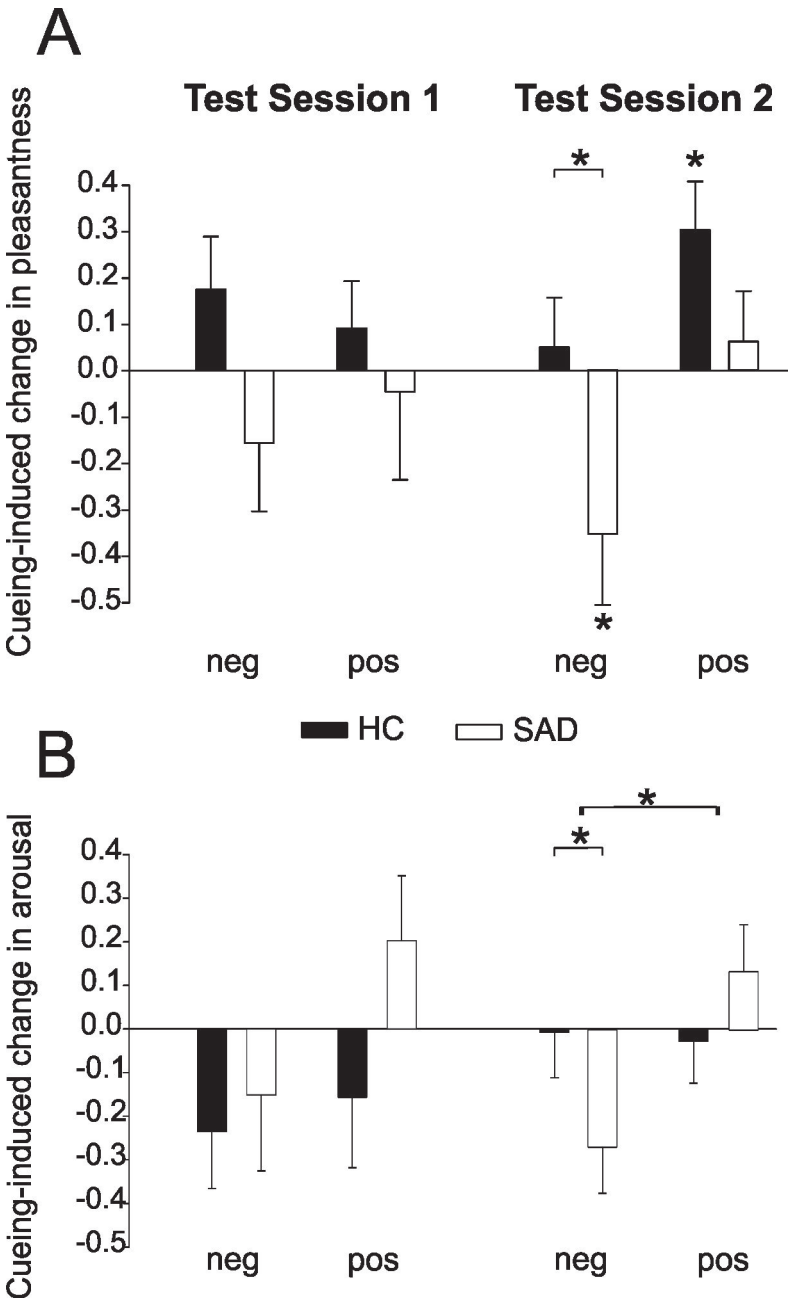
734 **Fig. 2.** Cueing-induced changes in ratings of pleasantness **(A)** and arousal **(B)** in Test Session
735 1 and Test Session 2 in healthy (HC, black bars) and socially anxious participants (SAD,
736 white bars). The cueing-induced change was calculated as the difference between
737 pleasantness/arousal ratings of pictures that were cued and pictures that were not cued during
738 sleep after learning. Thus, positive values indicate that cueing increased the subjectively rated
739 pleasantness/arousal of pictures whereas negative values indicate that cueing reduced the
740 subjectively rated pleasantness/arousal. **(A)** Cueing during post-learning sleep did not affect
741 ratings of pleasantness in Test Session 1 but it significantly modulated ratings of pleasantness

one week later. More specifically, memory cueing reduced the rated pleasantness of pictures previously associated with a negative word in socially anxious participants but not in healthy participants; and cueing increased the subjectively rated pleasantness of pictures previously being associated with a positive word in healthy but not SAD participants. **(B)** Cueing during post-learning sleep did also not affect arousal ratings in Test Session 1. One week later in Test Session 2, memory cueing reduced arousal ratings of pictures previously associated with a negative word in socially anxious participants but not in healthy participants.

Fig. 3. Time-frequency plots indicate the difference in the subsequent memory effect (SME, later remembered minus later forgotten stimuli) between socially anxious (SAD) and healthy participants (HC) for positive and negative cues. **(A)** The SME for positive cues did not differ between both groups. **(B)** Socially anxious participants as compared to healthy controls showed a higher SME to negative cues in the theta frequency range (i.e. 5 – 8 Hz, temporal electrode 41 is indicated here). **(C)** The topographical distribution of the difference in the SME between SAD and HC is indicated for mean activity in the theta frequency band 600 - 800 ms after cue onset. The difference in the SME was most pronounced in a cluster over the left frontal, temporal and parietal cortex ($P = 0.002$, corrected for multiple comparisons). (**D, E**) Analysing the SME for negative cues separately in socially anxious and healthy participants revealed a positive cluster of electrodes mainly located over the left temporal and parietal cortex in SAD (**D**, $P = 0.01$, corrected for multiple comparisons) and a negative cluster over the left frontal and temporal cortex in healthy controls (**E**, $P = 0.01$, corrected for multiple comparisons). T-values are indicated in C-E and significant electrodes ($P < 0.05$) are highlighted.







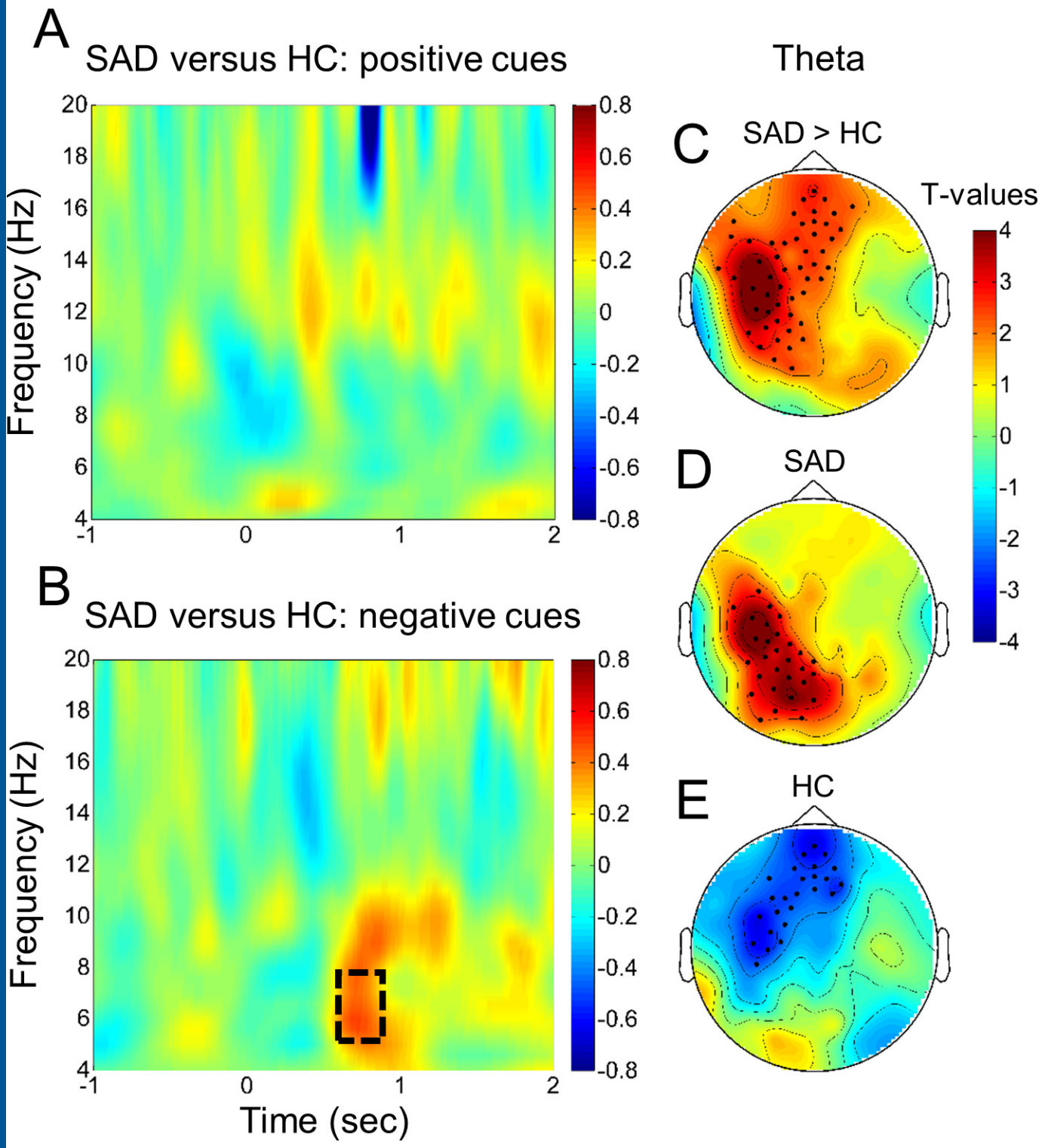


Table 1. Participants' data

	Healthy controls	**SAD**
	mean ± SEM	mean ± SEM
Age	13.11 ± 0.57	13.38 ± 0.58
Intelligence Quotient	109.54 ± 3.35	113.81 ± 3.52
SAD Questionnaires		
SASC-R-D FNE	15.00 ± 0.95	24.31 ± 2.88**
SASC-R-D SAD	18.62 ± 1.12	32.31 ± 2.56***
SASC-R-D FNE/ Parents	12.77 ± 0.95	28.58 ± 2.47***
SASC-R-D SAD/ Parents	16.23 ± 1.30	32.08 ± 2.80***
SPAIK	7.23 ± 0.97	26.38 ± 3.90***
Depression Interview CDRS-R		26.00 ± 3.34

Participants' data on age, intelligence quotient (IQ) as well as the results of standardized questionnaires assessing social anxiety, i.e. the „Sozialphobie und Angstinventar für Kinder" (SPAIK) and the „Social Anxiety Scale for Children Revised"–Child and Parents version (SASC-R-D). All parameters are given in mean (± SEM) and statistical differences between groups are indicated (**$p < 0.01$; ***$p < 0.001$).

Table 2. Sleep parameters

	Absolute time (in min)			Percentage of time (in %)		
	Healthy Controls	**SAD**		**Healthy Controls**	**SAD**	
	mean ± SEM	mean ± SEM	p-values	mean ± SEM	mean ± SEM	p-values
Sleep latency	19.11 ± 3.67	15.36 ± 3.53	0.47			
REMS latency	181.33 ± 20.57	151.77 ± 11.36	0.21			
Non REMS N1	21.42 ± 5.57	24.80 ± 4.81	0.65	4.46 ± 1.26	4.63 ± 0.82	0.91
Non REMS N2	241.22 ± 5.88	231.85 ± 13.91	0.55	48.53 ± 1.45	44.21 ± 2.55	0.16
Non REMS N3	143.11 ± 13.13	166.23 ± 15.49	0.27	28.27 ± 2.00	31.47 ± 2.60	0.35
REMS	94.44 ± 6.43	103.64 ± 7.64	0.37	18.74 ± 1.02	19.69 ± 1.45	0.60
TST	500.19 ± 14.32	526.51 ± 14.26	0.21			

Sleep parameters for healthy and socially anxious (SAD) participants are given in mean ± SEM of absolute time in minutes and percentage of total sleep time; REMS = rapid eye movement sleep, TST = total sleep time.

Table 3 Memory retention and affective ratings

	Healthy controls		SAD	
	Cued	Uncued	Cued	Uncued
	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
Encoding				
Positive	71.22 ± 3.17		72.66 ± 2.79	
Negative	73.85 ± 2.72		73.56 ± 2.61	
Memory retention				
Test Session 1				
Positive	105.93 ± 2.57	100.68 ± 1.39	103.16 ± 1.60	98.48 ± 2.15
Negative	100.55 ± 1.42	99.68 ± 1.59	101.96 ± 2.24	100.44 ± 2.58
Test Session 2				
Positive	86.37 ± 3.94	94.14 ± 2.94	83.55 ± 4.55	84.56 ± 2.37
Negative	82.96 ± 3.23	87.92 ± 3.57	82.53 ± 4.14	76.94 ± 3.88
Arousal				
Test Session 1				
Positive	2.57 ± 0.31	2.72 ± 0.31	2.78 ± 0.37	2.58 ± 0.36
Negative	2.55 ± 0.26	2.78 ± 0.27	3.27 ± 0.43	3.42 ± 0.42
Test Session 2				
Positive	2.79 ± 0.35	2.82 ± 0.34	3.79 ± 0.39	3.66 ± 0.36
Negative	2.76 ± 0.34	2.76 ± 0.37	3.81 ± 0.36	4.07 ± 0.34
Pleasantness				
Test Session 1				
Positive	5.75 ± 0.26	5.66 ± 0.28	5.62 ± 0.25	5.67 ± 0.19
Negative	5.08 ± 0.26	4.90 ± 0.23	4.77 ± 0.20	4.93 ± 0.22
Test Session 2				
Positive	5.80 ± 0.26	5.50 ± 0.28	5.30 ± 0.24	5.24 ± 0.20
Negative	5.16 ± 0.30	5.11 ± 0.26	4.70 ± 0.14	5.05 ± 0.18

Memory retention and affective ratings for cued and uncued stimuli at Encoding, Test Session 1 and Test Session 2 in socially anxious and healthy participants. Memory retention is given in percent relative to memory performance in the Learning Session. Subjective emotional arousal and pleasantness are assessed using a 9-point scale (1 = very unpleasant, 5 = neutral, 9

= very pleasant; 1 = not arousing at all, and 9 = very arousing, respectively). All parameter are given in mean (±SEM).